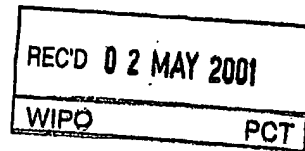


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I, MARIA LEWIS, TEAM LEADER EXAMINATION SUPPORT AND SALES hereby certify that annexed is a true copy of the Provisional specification in connection with Application No. PQ 6733 for a patent by STARCH AUSTRALASIA LIMITED filed on 06 April 2000.

I further certify that the name of the applicant has been amended to PENFORD AUSTRALIA LIMITED pursuant to the provisions of Section 104 of the Patents Act 1990.



WITNESS my hand this
Twenty-sixth day of April 2001

M. Lewis

MARIA LEWIS
TEAM LEADER EXAMINATION
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PROVISIONAL SPECIFICATION

Invention Title:

Starch sub-types and lipid metabolism

The invention is described in the following statement:

Technical Field

The present invention relates to fat or lipid metabolism and diets, particularly diets high in resistant starch.

Background Art

5 Insulin resistance is recognised as the underlying cause of a cluster of metabolic diseases known as Syndrome X or the Metabolic Syndrome. These diseases include non-insulin dependent diabetes mellitus, obesity, dislipidemias, hypertension and coronary heart disease. The factors which contribute to the onset and development of insulin resistance have not been
10 fully elucidated but the type of fat and/or carbohydrate in the diet have been exposed as crucial factors.

 Two polymers of glucose, amylose and amylopectin, constitute dietary starch. Amylose is a linear polymer of glucose residues linked by $\alpha(1-4)$ bonds whereas amylopectin is a branched polymer of glucose residues linked
15 by $\alpha(1-4)$ and $\alpha(1-6)$ bonds. Ingestion of a diet containing elevated level of amylose starch causes lower postprandial glycemia and insulinemic responses than consumption of a diet containing amylopectin starch.

 The rapid and prolonged rise in plasma insulin and glucose concentrations which accompany amylopectin starch ingestion has been
20 postulated to be detrimental to whole body insulin sensitivity in the long term. In humans, consumption of foods which cause a large rise in postprandial plasma glucose concentration is associated with an increased concentration of free fatty acids in the plasma. This increase in plasma free fatty add concentration causes a decrease in glucose oxidation, presumably
25 via the glucose-fatty acid cycle, which may impair insulin sensitivity. Postprandial hyperinsulinemia and hyperglycemia have also been shown to decrease glucose uptake through a decrease in GLUT 4 mRNA and protein abundance. In addition, hyperinsulinemia and/or hyperglycemia may be responsible either for a defect in GLUT 4 translocation or interference with
30 signal transduction of the insulin receptor.

 The reduction in postprandial plasma insulin and glucose concentrations which accompany high amylose starch consumption are due to its structural properties and the presence of resistant starch. Resistant starch is that fraction of starch which is resistant to digestion in the small
35 intestine, thereby restricting the quantity of glucose entering the bloodstream, and passes to the large bowel for fermentation. Indeed, in rats'

consumption of a high amylose, or high resistant starch, diet maintains insulin sensitivity in the long-term in relation to high amylopectin starch and glucose-based diets which are low in resistant starch.

Dietary fibre is defined as measured by the AOAC, International
5 (Association of the Official Analytical Chemistry) Total dietary fiber in foods: enzyme-gravimetric method (Method 985.29). Assoc. Off. Anal. Chemists, Official Methods of Analysis, 16th Ed, Arlington VA, USA, 1995.

The present inventors have determined similar differences in
postprandial blood parameters are present in humans consuming diets with
10 different resistant starch contents and identified changes in the carbohydrate/fat oxidation ratio which may occur due to the ingestion of resistant starch.

Disclosure of Invention

In a first aspect, the present invention consists in a method of
15 enhancing fat utilisation in a diet of an individual, the method comprising consuming in a given meal at least 5 gram of resistant starch with the fat to be utilised.

Preferably, the fat is a saturated fat, a mono-unsaturated fat, a poly-unsaturated fat, an omega-3 fat, or an omega 3-6 fat. Further vegetable
20 triglycerides relevant to this application include those obtained from seeds, beans, fruits, nuts and other plant materials, often obtained by mechanical expelling and/or solvent extraction. Examples which are particularly suitable for use in the present invention are sunflower oil including high and mid oleic varieties, soybean oil, cottonseed oil, canola or rapeseed oil including
25 low linolenic and other modified varieties, flax or linseed oil including high linolenic varieties [Linola], maize or corn oil, olive oil, peanut oil, rice bran oil, palm oil and fractionated palm oils, palm kernel oil, coconut oil and the like.

Triglycerides of animal origin can be used in the present invention and
30 include those obtained from milk and from the processing of cattle, sheep and fish.

As used in this specification, "resistant starch" includes those forms defined as RS1, RS2, RS3 and RS4 as defined in Brown, McNaught and Moloney (1995) Food Australia 47: 272-275. Either modified or unmodified
35 resistant starches or mixtures thereof can be used in the present invention.

Respiratory Quotient (RQ) is the molar ratio of carbon dioxide (CO_2) produced to oxygen (O_2) consumed and this ratio varies depending on the energy source being utilised by the body. RQ when oxidising carbohydrate as the sole energy source is theoretically 1.00, RQ when oxidising lipids as the sole energy source is theoretically 0.71. Mixed diets will produce RQs which vary between these two theoretical values.

Preferably, the resistant starch is derived from maize, sorghum, rice, barley, oats, triticale, wheat, legumes, potato, or banana starches. As the amylose content of some starches appears to be related to the resistant starch content, one preferred embodiment is the use of starches having an amylose content of at least 40% (w/w). Resistant starch obtained or derived from maize starch has been found to be particularly suitable for the present invention. In many starch containing plants, the amylose content does not need to increase to the high levels found in maize in order for them to demonstrate the properties of resistant starch. These properties are likely to be found in wheat [+35% amylose], banana and barley [+30% amylose]; potato, legumes and rice [+27% amylose]. The amount of resistant starch can be demonstrated by the resistance of the starch granule or starch derived material to attack by amylases, irrespective of its amylose content. Although the amylose content can act as an indicator of whether the starch granule will exhibit this property of resistance to amylolysis.

Maize starches having an amylose content of at least 70% (w/w), at least 80% (w/w) or at least 90% (w/w) are preferred as these starches contain high levels of starch granules forming resistant starch.

The starch can chemically, physically, and/or enzymically treated or modified to enhance the yield or alter the resistant starch present. Chemical modification can be in the form of oxidation, cross-bonding, etherification, esterification, acidification, dextrinisation, and mixtures thereof. One preferred physical treatment is heat-moisture treatment to enhance or increase the resistant starch content of the starch. Another treatment is by solvent extraction to remove fats and/or minerals from the starch.

Preferably, the meal contains at least 10 grams of resistant starch or at least 5 grams higher than a comparable meal containing a high quantity of readily digestible starches. It has been found that the consumption of at least 15 grams, preferably at least 20 grams, and more preferably around 30 grams total resistant starch per day with meals provides an improved fat

metabolism of fat, namely increased oxidation of dietary fats and/or mobilisation and utilisation of stored fats. in an individual.

In a second aspect, the present invention consists in a method of lowering the incidence of obesity in an individual through the stimulation of increased levels of fat oxidation, the method comprising placing the individual on a high carbohydrate diet, rich in resistant starch to stimulate increased levels of fat oxidation in the individual.

In a third aspect, the present invention consists in a method of lowering the incidence of non-insulin dependent diabetes mellitus in an individual, the method comprising placing the individual on a high carbohydrate diet, rich in resistant starch, to stimulate increased levels of fat oxidation in the individual.

Preferably, the high carbohydrate diet rich in resistant starch provides approximately 50% (it may be higher or lower) of the available calories from carbohydrate, with at least 5 grams, preferably 10 grams,, more preferably at least 20 gram, even more preferably at least 25 gram, and most preferably at least 30 gram resistant starch per day. The consumption of at least 5 grams of resistant starch, preferably at least 10 grams in a single meal will also have a beneficial effect on by increasing fat oxidation.

Individuals predisposed to obesity or non-insulin dependent diabetes mellitus can be placed on the diet as a means of preventing or delaying the onset of the disease state. Also individuals already suffering from these conditions can effect these changes to the diet as part of the treatment regime.

One form of resistant starch particularly suitable for the present invention is starch containing resistant starch. Preferably, the starches have an amylose content of at least 40% (w/w), although the amylose content of the starch may vary depending on the plant species from which the starch has been obtained. In a preferred form the starch is from maize having an amylose content of at least 70% (w/w), at least 80% (w/w) or at least 90% (w/w). The starch can also be chemically, physically, or enzymically treated or modified. Chemical modification can be by oxidation, cross-bonding, etherification, esterification, acidification, dextrinisation, or mixtures thereof. Physical modification includes heat-moisture treatment,

Preferably the resistant starches are derived or obtained from maize (corn). It will be appreciated, however, that other sources of resistant starch

could be used in the present invention. Examples include cereals like sorghum, wheat, barley, oats, triticale, maize and rice, tubers like potatoes and tapioca, legumes such as peas, and others including starches derived from conventional inbred breeding techniques or from genetically modified plant species.

5 Starches can also be treated to enhance the resistant starch content by a number of physical or chemical means. One preferred means is to heat-treat starch in the presence of moisture (heat-moisture treatment) which can be achieved by a number of processes including heating under negative, 10 atmospheric or positive pressure under elevated moisture, or cycling techniques through different temperatures and pressures. Heating can be in the order of 100 to 180°C, preferably around 120 to 150°C and moisture levels of 10 to 80%, preferably 20 to 60%. Repeated autoclaving and rapid cooling can also be used to increase the resistant starch content of starches. It will 15 be appreciated that these processes and conditions can be changed to achieve the desired increase in the level of resistant starch in the starch being treated.

Treatment can also be by solvent extraction to remove fats and/or minerals from the starch.

In WO 94/03049 and WO 94/14342, high amylose starches are 20 disclosed which are resistant starches and include maize starch having an amylose content of 50% (w/w) or more, particularly 80% (w/w) or more, rice starch having an amylose content of 27% (w/w) or more, or a wheat starch having 35% (w/w) or more. Furthermore, particular granular size ranges of starches having an amylose content of 50% or more and enhanced resistant 25 starch content, these starches including maize, barley, and legumes. This invention is not, however, limited to these forms of resistant starch. For example, other forms of resistant starch are derived from sources such as bananas and tubers such as potatoes and modified forms thereof.

Chemical modifications, such as oxidation, cross-bonding, 30 etherification, esterification, acidification, dextrinisation and the like are well known in this art as being suitable chemical treatments. Similarly, other modifications can be induced physically, enzymically or by other means well known to those skilled in the art.

It may also be useful to modify the degree of enzyme susceptibility of 35 the resistant starch by altering the conformation or structure of the starch. Examples include acid or enzyme thinning and cross bonding using di-

functional reagents, heat/moisture treatment and thermal annealing. Modification of the starch may also be carried out by manipulation of the crystalline nature of the starch. Such modification methods are known to the art and starches produced by these methods would be suitable for the present invention.

The present invention is applicable for animals and humans by manipulating the diet through feed, food, supplements and pharmaceuticals. Although resistant starch can be added to foods, other dietary components such as fats, proteins, particularly proteins resistant to digestion and termed "by-pass proteins", minerals, particularly calcium, iron, zinc, to ensure optimal physiological performance or utilisation.

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

In order that the present invention may be more clearly understood, preferred forms will be described with reference to the following examples and drawings.

20 Brief Description of Drawings

Figure 1. Fasting blood parameters in response to resistant starch in the diet. Antecubital blood samples were taken 0 and 14 days after commencing a DS or RS diet. Results are expressed as mean \pm SEM (n = 12 for DS, n = 11 for RS). * p < 0.03 for a difference from DS at day 0. ** p < 0.02 for a difference from RS at day 0.

Figure 2. Postprandial blood parameters. Two weeks after commencing a DS or RS diet (day 14), subjects returned for a follow-up fasting blood sample and a 3 hour meal test. The test meal consisted of 60 g breakfast cereal, 250 mL Lite White milk, 1 slice of bread (toasted), 1 muffin (toasted), 10 g of Canola margarine and 20 g of jam. Results are expressed as mean \pm SEM (n = 12 for DS, n = 11 for RS).

Figure 3. Change in RQ in response to resistance starch in the diet. Two weeks after commencing a DS or RS diet (day 14), subjects returned for a follow-up fasting blood sample and a 3 hour meal test. The test meal consisted of 60 g breakfast cereal, 250 mL Lite White milk, 1 slice of bread (toasted), 1 muffin (toasted), 10g of Canola margarine and 20 g of jam.

Results are expressed as mean \pm SEM (n = 12 for DS solid circles, n = 11 for RS, open circles). *p < 0.03 for difference from the RS group at the same time point.

Modes for Carrying Out the Invention

5 METHODS

Twenty-four healthy males (19 - 34 years of age) participated in the present study. Approval for this work was granted by the University of Wollongong Human Ethics Committee and full written consent was obtained from all subjects prior to commencement of the trial.

10 Subjects were randomly divided into two groups. The first group received a traditional starch (TS) diet, low in resistant starch, whereas the second group received a Hi-maize™ (HM) diet, high in resistant starch. The TS diet consisted of standard commercially available products whereas the HM diet consisted of commercially available product containing [Hi-maize™
15 (Table 1). For the TS group, mean and SEM values for age, height and weight were 22.3 ± 0.6 years, 180 ± 3.1 cm. and 73.5 ± 3.7 kg, respectively. For the HM group, mean and SEM values for age, height and weight were 23.5 ± 0.6 years, 185 ± 1.8 cm, and 74.1 ± 2.4 kg, respectively.

All subjects were requested to eat at least 60 g breakfast cereal, 4 slices
20 of white bread, and 2 muffins per day plus 3 pasta meals (125 g servings) per week for 14 days. An excess of these foods was provided such that subjects could exceed the intake guidelines if necessary as all participants were exercising regularly (4-8 times per week). All subjects were advised not to eat foods containing a significant amount of resistant starch (eg. legumes,
25 green bananas and bismati rice) during the study in effort to control the 'background' intake of resistant starch (ie. resistant starch from sources other than those provided as part of the study). All foods supplied to subject were donated by Buttercup Bakeries, Uncle Toby's Company Ltd, and New Zealand Starch Products on behalf of Starch Australasia Ltd.

30 Before commencing the allotted diet (day 0), a fasting venous blood sample (antecubital) was taken from each subject followed by a diet history interview and thorough explanation of the dietary guidelines for the study. Two weeks after commencing the diet (day 14), subjects returned for a follow-up fasting blood sample and a 3 hour meal test. The test meal was
35 either TS or HM, based upon the subject's diet over the two week study period, and consisted of 60g breakfast cereal, 250 ml Lite White milk, 1 slice

of bread (toasted), 1 muffin (toasted), 10 g of Canola margarine and 20 g of jam. Venous blood samples (antecubital) were taken 30, 60, 120 and 180 min post-ingestion of the test meal. All blood samples were subsequently analysed for serum glucose, serum insulin, plasma cholesterol, plasma total
5 lipid and plasma free fatty acid concentration. In addition, respiratory quotient (RQ) measurements were taken at 0, 60, 120 and 180 min after ingestion of the test meal using a Datex Deltatrac II (Helsinki, Finland).

Serum glucose concentration was determined using a glucose oxidase, peroxidase colorimetric assay kit (Boehringer Mannheim, Germany). Serum
10 insulin concentration was estimated using Linco (St Louis, USA) human radioimmunoassay kits. Plasma free fatty acids (FFA) were measured using a NEFA (non-esterified fatty acids) C kit (Wako Pure Chemicals Inc. Japan). Plasma cholesterol and triglyceride concentrations were determined using colorimetric assay kits supplied by Boehringer Mannheim (Germany). All
15 assays were conducted according the manufacturer's instructions.

Table 1. Resistant starch (RS) content of foods consumed (% w/w)

	Traditional starch group (TS)		Hi-maize™ group (HM)	
	Product	RS content	Product	RS content
Cereal	Uncle Toby's MaxNRC	0.7	Uncle Toby's Grinners	3.4
Bread	Buttercup Super Sandwich Maker	0.8	Buttercup Wonder White	2.9
Muffins	Buttercup English Muffins	0.8	Wonder White Muffins	1.6
Pasta	Vetta pasta Spirals	<0.1	Hansell Pasta Spiral	1.5
	TOTAL per meal	2.4		9.4

RESULTS

- 5 Of the 24 subjects recruited, one subject from the HM group was found to be insulin resistant according to World Health Organisation (WHO) criteria and was eliminated from the study. Total energy intake and macronutrient composition of the diet did not significantly differ between the TS and HM groups (Table 2).

10

Table 2. Actual nutrient intake during the dietary intervention as assessed from diet history records and subject's daily food check list. Values indicated as a percentage represent the percentage of total calorie intake.

	Traditional starch group	Hi-maize™ group
Energy intake (kJ/d)	13871 ± 3500	13258 ± 3100
Carbohydrate (%)	53 ± 1.0	57 ± 1.4
Resistant starch (g/d)		
Protein (%)	17 ± 0.3	16 ± 0.2
Total Fat (%)	27 ± 0.3	24 ± 0.2
Saturated fat (%)	12 ± 0.1	11 ± 0.1
Mono-unsaturated fat (%)	10 ± 0.1	8 ± 0.1
Poly-unsaturated fat (%)	5 ± 0.1	5 ± 0.04

15

Fasting insulin, glucose, lipid and cholesterol concentrations did not change between day 0 and day 14 irrespective of the diet (Figure 1). Serum FFA concentration, however, decreased between day 0 and day 14 in both the TS and HM groups. There was a trend for the fasting FFA concentration to be higher in the HM group than the TS group after 14 days on the specified diet although this did not reach statistical significance.

The TS and HM groups showed no difference in postprandial insulin, glucose, lipid, FFA or cholesterol responses at any time point measured (Figure 2). Plasma FFA concentration tended to be higher in the HM group than the TS group 2 h after ingestion of the test meal but this relationship did not reach statistical significance.

There was no difference in fasting RQ values between the TS and HM groups (data not shown). RQ values ranged between 0.83 and 0.91 and were plotted as ΔRQ which represents the difference between the RQ at each time point and that at 0 min (Figure 3). The ΔRQ at 60 and 120 min after meal ingestion showed no difference between the TS and HM groups. After 180 min, however, the ΔRQ for the HM group was approximately 50% of that for the TS group.

DISCUSSION

The decrease in fasting FFA concentrations which occurred between day 0 and day 14 in both HM and TS groups was due to the consumption of a high carbohydrate diet by all subjects during the study. The lack of difference in postprandial insulin and glucose concentrations between the TS and HM groups may be due to the cohort of subjects used in this study, all of whom were young, highly active and insulin sensitive. It is anticipated that subjects will display differences in post prandial insulin and glucose concentrations with diets that change the type and ratio of resistant starch and lipid. In addition, the difference in the total resistant starch content between the HM and TS diets was relatively low. The meal used for the acute assessment contained approximately four times as much resistant starch as the TS diet (28.2% versus 7.2% (w/w), respectively).

In absolute terms, carbohydrate was the primary source of energy at 1, 2 and 3 hours post-meal ingestion as RQ values ranged from 0.90 to 0.92. The decrease in RQ which was observed in the HM group relative to the TS group 3 hours after eating represents an increase in fat oxidation. Although the magnitude of this decrease in RQ (0.05 units) seems small, it accounts for

a large difference in fat oxidation. For example, if subjects in the TS group were oxidising 50% fat and 50% carbohydrate, the observed decrease in RQ would mean that subjects in the HM group were oxidising 67% fat and 33% carbohydrate. This substantial difference in fuel utilisation is of particular
 5 interest as there was no difference in postprandial blood parameters. It is important to note, however, that fat oxidation increased in the HM group directly following the trend to higher FFA concentrations at 2 h and may be responsible for the absence of any difference in plasma FFA concentration from the TS group at 3h.

10 Resistant starch content of the diet had no effect on fasting or postprandial glucose, insulin, FFA, cholesterol or total lipid concentrations. It is anticipated that subjects will display differences in post prandial FFA, cholesterol and total lipid concentrations with diets that change the type and ratio of resistant starch and lipid. Resistant starch consumption did,
 15 however, cause an acute increase in fat oxidation. In addition, consumption of a high carbohydrate diet, irrespective of resistant content, lowered fasting plasma FFA concentrations. Taken together, these results indicate that a high carbohydrate diet, rich in resistant starch may be beneficial for those who suffer metabolic diseases in which plasma FFA oversupply is
 20 symptomatic such as obesity and non-insulin dependent diabetes mellitus.
 SUMMARY

From data obtained in rodents, it has been established that the type of complex carbohydrate in the diet influences postprandial insulinemic and glycemic responses and, therefore, insulin sensitivity. Starches containing
 25 little resistant starch (RS) cause prolonged elevation in plasma glucose and insulin concentrations with respect to those starches high in resistant starch. The present study was performed in order to determine if similar differences in postprandial blood parameters are present in humans in response to different resistant starch content of the diet and identify any changes in the
 30 carbohydrate/fat oxidation ratio which may occur due to the ingestion of resistant starch. Two groups of healthy males (age 18-34 years) consumed a high carbohydrate diet containing either traditional starch (TS) products low in resistant starch or Hi-maize™ (HM) products high in resistant starch content for two weeks. Fasting blood samples were taken before
 35 commencement of the diet and again at its completion. At the completion of the diet periods, subjects underwent a meal test in which the test meal

corresponded to their diet during the intervention period. RQ measurement and blood samples were taken post-meal ingestion to be analysed for glucose, insulin, free fatty acid (FFA), cholesterol and total lipid concentration. A significant decrease in fasting FFA concentrations was observed for both the TS and HM groups between the beginning and the completion of the diet period (eg for the TS group, 0.7 ± 0.05 mM at day 0 vs 0.5 ± 0.02 mM at day 14, $p < 0.03$). There were no significant differences in fasting or post-prandial glucose, insulin, cholesterol, or total lipids between the TS and HM groups. There was a trend for FFA to be higher in the HM group than the TS group 2 h after test meal ingestion (0.4 ± 0.02 mM vs. 0.3 ± 0.02 mM, not significant). At 3 hours the Δ RQ for the HM group (0.04 ± 0.01) was approximately 50% of that for the TS group (0.09 ± 0.02 ; $p < 0.01$). This data provides evidence that a diet high in resistant starch causes an acute shift in fuel utilisation that favours fat oxidation over carbohydrate oxidation with the consumption of elevated levels of dietary resistant starch.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

Dated this sixth day of April 2000

Perfom Australia Limited
Starch Australasia Limited

Patent Attorneys for the Applicant:

F B RICE & CO



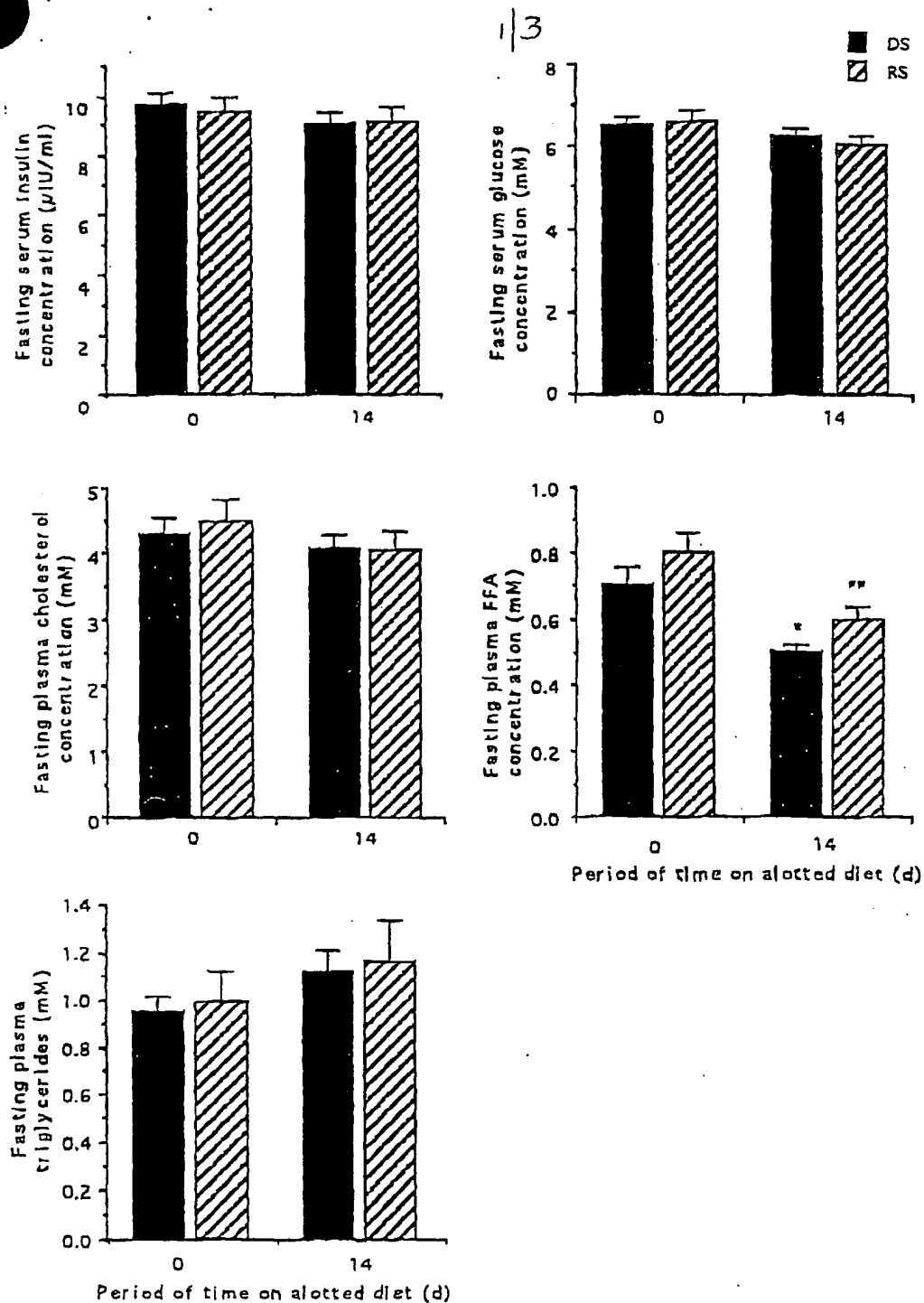


Figure 1. Fasting blood parameters in response to resistant starch in the diet. Antecubital blood samples were taken 0 and 14 days after commencing a DS or RS diet. Results are expressed as mean \pm SEM ($n = 12$ for DS, $n = 11$ for RS). * $p < 0.03$ for a difference from DS at day 0. ** $p < 0.02$ for a difference from RS at day 0.

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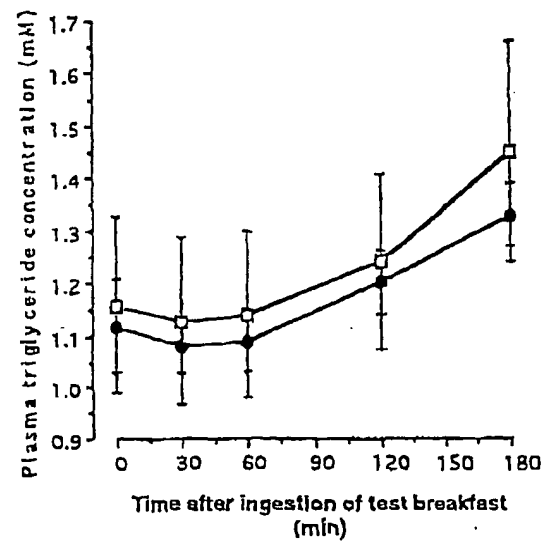
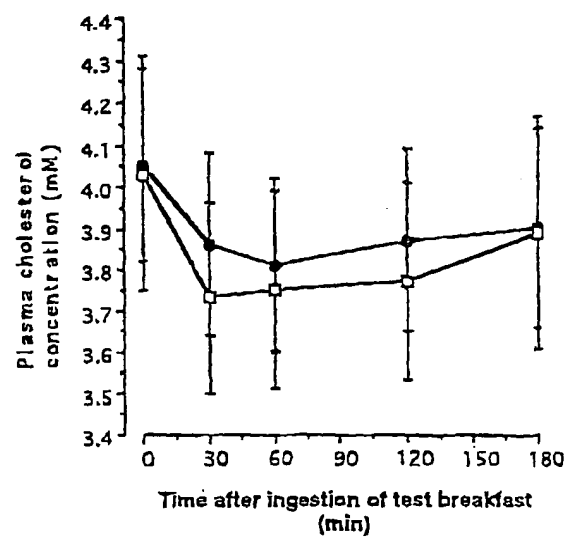
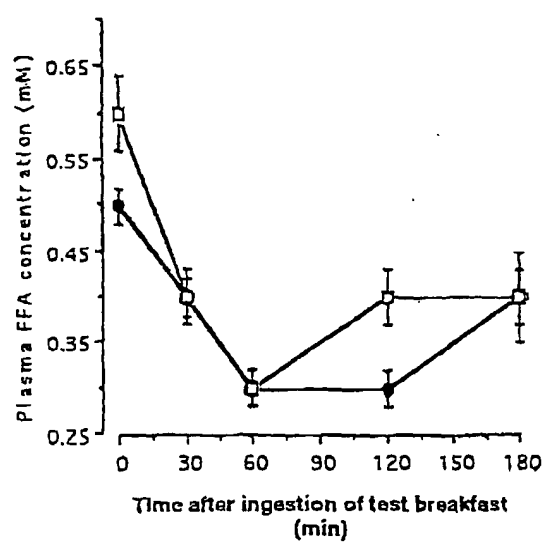
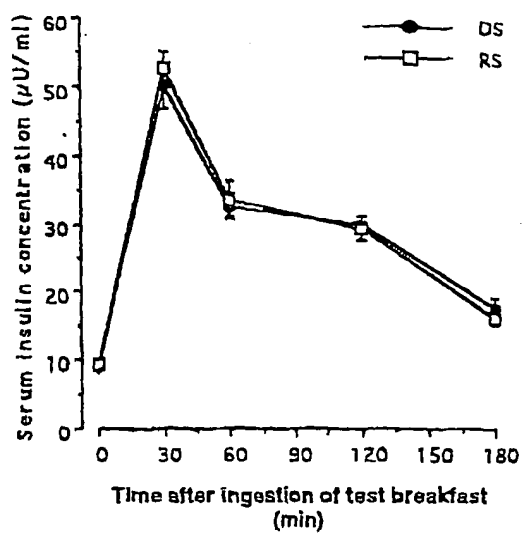
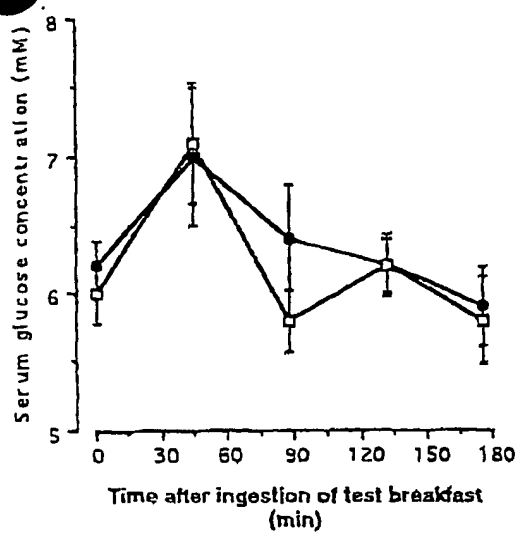


Figure 2. Postprandial blood parameters. Two weeks after commencing a DS or RS diet (day 14), subjects returned for a follow-up fasting blood sample and a 3 hour meal test. The test meal consisted of 60 g breakfast cereal, 250 ml Lite White milk, 1 slice of bread (toasted), 1 muffin (toasted), 10 g of Canola margarine and 20 g of jam. Results are expressed as mean \pm SEM (n = 12 for DS, n = 11 for RS).

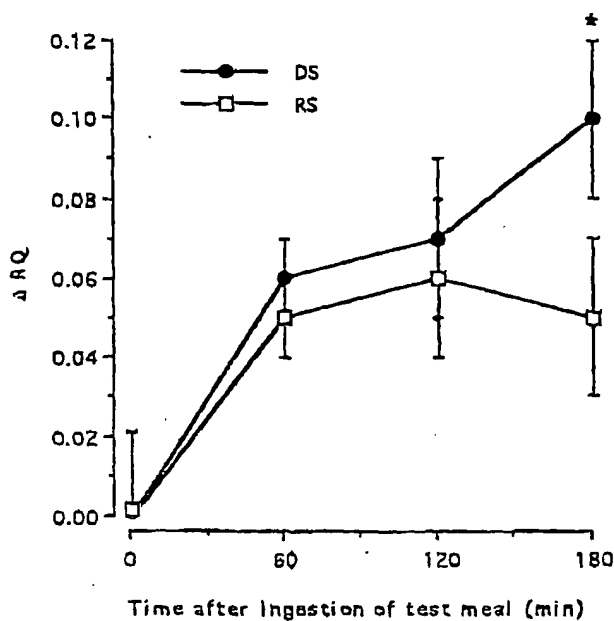


Figure 3. Change in RQ in response to resistant starch in the diet. Two weeks after commencing a DS or RS diet (day 14), subjects returned for a follow-up fasting blood sample and a 3 hour meal test. The test meal consisted of 60 g breakfast cereal, 250 ml Lite White milk, 1 slice of bread (toasted), 1 muffin (toasted), 10 g of Canola margarine and 20 g of jam. Results are expressed as mean \pm SEM ($n = 12$ for DS solid circles, $n = 11$ for RS, open circles). * $p < 0.03$ for difference from the RS group at the same time point.